

The Impact of the Host on Fungal Infections

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ABSTRACT

Outcomes of fungal infections in immunocompromised individuals depend on a complex interplay between host and pathogen factors, as well as treatment modalities. Problems occur when host responses to an infection are either too weak to effectively help eradicate the pathogen, or when they become too strong and are associated with host damage rather than protection. Immune reconstitution syndrome (IRS) can be generally defined as a restoration of host immunity in a previously immunosuppressed patient that becomes dysregulated and overly robust, resulting in host damage and sometimes death. IRS associated with opportunistic mycoses presents as new or worsening clinical symptoms or radiographic signs consistent with an inflammatory process that occur during receipt of an appropriate antifungal, and that cannot be explained by a newly acquired infection. Because there are currently no established tests or biomarkers for IRS, it can be difficult to distinguish from progression of the original infection, although culture and biomarkers for the fungal pathogen or infection are typically negative during diagnostic workup. IRS was originally characterized in human immunodeficiency virus-infected patients receiving antiretroviral therapy, but has subsequently been described in solid-organ transplant recipients, neutropenic patients, women in the postpartum period, and recipients of tumor necrosis factor- α inhibitor therapy. In each of these cases, recovery of the host's immunity during treatment of an initial infection results in a powerful proinflammatory environment that overshoots and leads to host damage. Optimal management of IRS has not been established at present, but often involves treatment with a corticosteroid or other anti-inflammatory compounds. This article uses a number of patient cases to explore the intricacies of diagnosing and managing a patient with IRS, as well as the other extreme, namely patients who are so immunocompromised without immune recovery that they essentially become breeding grounds for a wide range of opportunistic pathogens, often simultaneously.

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Primary treatment of invasive fungal infections (IFIs) can and too often does fail, despite improvements in diagnostic tools and an increased number of antifungal options. Potential causes of failure include intrinsic drug resistance, wrong

diagnosis, a mixed infection, failure to achieve and/or maintain adequate drug concentrations at the site of infection, drug toxicities, development of resistance, and various host-related factors.¹ Clinicians and investigators increasingly understand that the outcome of IFIs or other serious infections depends not simply on the pathogen or host involved, nor only on the antifungal regimen used to treat the infection, but on a complex interplay between these factors. This review examines the impact of the host on the outcome of fungal infections, with a particular focus on immune reconstitution syndrome (IRS; also known as immune reconstitution inflammatory syndrome [IRIS], immune restoration disease, or paradoxical reactions/responses).^{2,3} IRS represents an overly robust host response to infection that leads to host damage. This review also briefly examines the other end of the spectrum, when the host is so

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immunocompromised that he or she becomes susceptible to infection and disease or host damage caused by a wide range of pathogens, often acting simultaneously.

CASE 1

A 22-year-old woman presented with rejection of a 4-year-old kidney transplant. She received treatment with high-dose corticosteroids and muromonab-CD3 for the rejection episode, and later received additional corticosteroid treatment for acute pericarditis. Three months after treatment of the rejection episode, while on maintenance immunosuppression (tacrolimus, mycophenolate mofetil [MMF], and prednisone), the patient presented with a 1-week history of headaches, nausea, and fever. Her temperature was 37.2°F (99°C). A neurologic examination revealed bilateral clonus and papilledema, with normal mental status. Blood work demonstrates a normal complete blood count and high serum creatinine (4.0 mg/dL; normal range in women, 0.7 to 1.2 mg/dL). A lumbar puncture was performed, and laboratory workup of the cerebrospinal fluid (CSF) shows an elevated white blood cell (WBC) count (100 cells/mm³; normal range, 0 to 5 cells/mm³), moderately low glucose level (43 mg/dL; normal range, 50 to 80 mg/dL), and a mildly elevated protein level (79 mg/dL; normal range, 15 to 45 mg/dL). The opening CSF pressure was high (400 mm H₂O; normal < 200 mm H₂O). CSF cultures grew *Cryptococcus neoformans*. Results of magnetic resonance imaging (MRI) of the head showed basilar meningitis, which was supported by a positive India ink test, and high cryptococcal antigen titers in CSF (1:256) and serum (>1:1,024).

The patient was initiated on liposomal amphotericin B (5 mg/kg/day for 20 days) and flucytosine induction dosed for renal dysfunction for 14 days) therapy, followed by consolidation with fluconazole (200 mg/day) therapy. Immunosuppressive therapy to prevent transplant rejection was continued, but with less aggressive dosing (MMF was discontinued, and both the tacrolimus and prednisone doses were reduced), in an attempt to clear the cryptococcal infection. A lumbar puncture taken 2 weeks later revealed a positive response to antifungal therapy and immunosuppression lowering: negative culture for *C neoformans*, negative India ink test, and opening pressure of 140 mm H₂O. The CSF antigen titer remained at 1:256. The patient's WBC count was reduced to 28 cells/mm³, from 100 cells/mm³.

After a very smooth initial clinical course, and 4 months after the diagnosis of *C neoformans* meningitis and while continuing fluconazole on maintenance therapy, the patient returned with new-onset of severe headaches. An MRI scan was performed and showed diffuse supra- and infratentorial leptomeningeal enhancement. The patient's CSF cryptococcal antigen titer was lowered from the prior measurement (1:16), but her CSF WBC count had increased to 100 cells/mm³. Her CSF cultures fail to grow *C neoformans* or any other pathogen.

Two weeks of liposomal amphotericin B therapy was again initiated, and added to the fluconazole maintenance therapy. The CSF cultures remain negative, but her head-

aches fail to improve. Fluconazole therapy was continued while dexamethasone treatment was initiated, and then tapered over 6 weeks. The patient completely rejected her kidney transplant and patient was started on dialysis. Tacrolimus and MMF were discontinued. One week after discontinuation of dexamethasone treatment, the severe headaches returned. Another lumbar puncture was performed, and both CSF cultures and cryptococcal antigen titer were negative. An MRI scan showed general improvement, with some continued meningeal enhancement. Dexamethasone therapy was again initiated and tapered over a 4-month period. The patient improved, and 2 years after discontinuing dexamethasone treatment for the second time, the patient was asymptomatic and ready to receive a second kidney transplant.

This case most likely represents IRS in a solid-organ transplant recipient with an initial fungal infection of the central nervous system successfully treated by antifungal therapy which included liposomal amphotericin B and a reduction in the potent immunosuppressive therapy used to prevent transplant rejection. The case could easily be diagnosed as failure of antifungal therapy or a recurrence of the initial infection. However, the negative culture results suggested that IRS may be the cause of the patient's subsequent problems and renal allograft loss. Subsequent sections of this article take a closer look at IRS in solid-organ transplant recipients and other at-risk patient populations, including definition and diagnosis, incidence and risk factors, and prevention or management strategies. The damage-response framework of microbial pathogenesis provides a foundation for understanding IRS, and is discussed before focusing on IRS itself.

THE DAMAGE-RESPONSE FRAMEWORK OF MICROBIAL PATHOGENESIS

Clinicians deal with disease and disease management. An infection may occur in a particular host or patient, but the clinician is principally focused on managing the disease following infection that changes homeostatic forces within the patient. The clinician's focus is on eradicating the pathogenic microorganism that is disturbing and destroying the patient. It is sometimes easy to forget that there is a patient involved, and that the strength of the patient's immunologic defenses can change over the disease course and sometimes overshoot and damage the patient, rather than protect against pathogen-initiated damage. The framework for understanding patient-pathogen interactions, and how reconstitution of a patient's immune system can result in counterproductive changes, was worked out in a series of articles by Casadevall and Pirofski.⁴⁻⁶ This framework forms the foundation for understanding IRS, and appreciation of IRS is essential for making decisions about the management of patients who deteriorate and exhibit signs of potential reinfection after initially showing response to antimicrobial therapy.

As outlined most recently in 2003, Casadevall and Pirofski postulated that microbial pathogenesis is the end result of a complex interaction between pathogen and pa-

tient (host), that this outcome is characterized by the degree of damage experienced by the patient, and that the damage may be attributed to either pathogen- or patient-related factors, or a combination of both.⁵ The reason for the increased appreciation of patient-related factors was driven by the appearance in the latter part of the 20th century of an increasing number of serious infectious diseases involving pathogens once considered relatively harmless or uncommon. In turn, the increased incidence of diseases caused by “opportunistic” microbes was related to the increasing pool of individuals with severely compromised immune systems. This pool developed in large part due to the acquired immunodeficiency syndrome (AIDS) epidemic and increasing numbers of patients with hematopoietic stem cell or solid-organ transplants, hematologic or solid cancers, or severe illness requiring prolonged treatment involving intravascular catheters or other invasive procedures.

Microbial-related damage to the patient obviously can occur when the patient’s immune system is so weakened that it cannot effectively eradicate the pathogen, allowing the pathogen to produce its tissue-damaging effects via a variety of potential means.^{5,6} A weakened immune system may also enable pathogens with low virulence to cause damage or to establish themselves in protected niches, whereas they would not be able to do so in immunocompetent hosts. Furthermore, a lowering in immune status can result in the transformation of preexistent but latent, colonizing, or non-disease-producing microbes into disease-producing pathogens. In each of these cases, a patient-related factor (lowered immune status) has enabled the pathogen to achieve the upper hand in its interaction with the patient. This is a generally understood and well accepted concept. However, what is sometimes less appreciated, is that the patient’s immune response to an invading microorganism can sometimes become unregulated or excessive, and inflammation or other immune-related responses can themselves cause tissue damage in the patient.

The ideal scenario for patient immunity is “the Goldilocks paradigm”: strong enough to eradicate or otherwise immobilize the invading pathogen, but not so strong that it causes patient damage (not too little and not too much but must get immune response just right). Casadevall and Pirofski⁵ illustrate this concept in their 2003 article by means of a parabolic curve of the damage-response framework, where either a weak or strong patient (host) response is associated with patient damage and (if weak or strong enough) death, and where a moderate patient response is associated with patient benefit. IRS represents the situation where the patient response is too strong, or at least becomes so during some later point in the clinical course.

IMMUNE RECONSTITUTION SYNDROME

Definition and Diagnostic Criteria for IRS

The concept of IRS was first popularized and characterized in patients with the human immunodeficiency virus (HIV) who were receiving antiretroviral therapy (ART),^{2,3} but IRS

has subsequently been recognized and received increasing attention in non-HIV immunocompromised patients, including transplant recipients and other patient populations at high risk for IFIs.^{3,7,8} Currently, there is no well-established biomarker or “gold standard” definition of IRS in HIV or other patient populations.^{2,7} However, widely accepted minimum criteria exist for the diagnosis of HIV-related IRS,² and Singh and Perfect⁷ have recently proposed diagnostic criteria for IRS associated with opportunistic mycoses.

According to Singh and Perfect,⁷ IRS should be considered in patients with a previously diagnosed IFI when *all three* of the following criteria are present:

- New appearance or worsening of clinical or radiologic manifestations consistent with an inflammatory process.
- Symptoms occurring during receipt of appropriate antifungal therapy that cannot be explained by a newly acquired infection.
- Negative results of cultures or stable or reduced biomarkers for the initial fungal pathogen during the diagnostic workup for the inflammatory process.

Clinical or radiologic manifestations consistent with an inflammatory process include contrast-enhancing lesions on neuroimaging studies (computed tomography [CT] or MRI); CSF pleocytosis (i.e., >5 WBCs per μL); increased intracranial pressure (opening pressure ≥ 200 mm H₂O), with or without hydrocephalus; histopathology showing granulomatous lesions; and unexplained hypercalcemia.⁷ To determine that the patient has received appropriate antifungal therapy, the clinician should take steps to exclude intrinsic or acquired drug resistance and ensure that antifungal drug concentrations are not suboptimal. It should be remembered that IRS is not a monolithic, rigidly defined entity, but rather a collection of localized and systemic reactions of varying degrees with both positive and negative features.

The patient in case 1 (described above) exhibited signs and symptoms consistent with an inflammatory process after treatment of *C neoformans* infection with liposomal amphotericin B plus flucytosine, followed by fluconazole, reduction of immunosuppressives and in the presence of negative culture results for *C neoformans*. Hence, the clinician should have had a heightened awareness of the possibility that IRS was the cause for subsequent difficulties, and proceeded accordingly. A high suspicion of IRS under appropriate conditions is important because occurrence of IRS is too often misinterpreted as a failure of antifungal therapy or as the presence of a new infection, leading to inappropriate and possibly counterproductive changes in the antimicrobial regimen.

Pathophysiologic Basis

The pathophysiologic basis of IRS may differ somewhat from patient to patient and for different underlying conditions, but it appears to generally involve a complex interplay of host and pathogen factors, and possibly antifungal drug factors. Host genetics may also play an important role, at least in some patients.⁹⁻¹¹ Host immune responses, by def-

initiation, are a key component of IRS. Cell-mediated immunity driven by T lymphocytes (especially T helper cells [T_H cells] and regulatory T cells [T_{regs}]) seems to play a particularly critical role,^{3,7,8} although there is increasing appreciation of the possible involvement of B lymphocytes and antibody-mediated or humoral immunity as well.^{8,12} Pathogen exposure causes activation of naive CD4 T lymphocytes (T-helper 0 [T_H0] cells) and their differentiation into either T_H1 or T_H2 cells, dependent on the cytokine milieu, which have distinct cytokine responses and effector functions.^{3,7,8} T_H1 cells are associated with proinflammatory responses, while T_H2 cells produce cytokines leading to anti-inflammatory and immunosuppressive responses. More recently, T_H17 cells and inducible T_{regs} have also been implicated in cell-mediated immunity to pathogen exposure and IRS, with T_H17 cells promoting proinflammatory responses and T_{regs} anti-inflammatory/immunosuppressive responses.

Infection control requires that proinflammatory responses (mediated by T_H1 and T_H17 cells) are not dominated by anti-inflammatory responses (mediated by T_H2 and T_{reg} cells). However, if the anti-inflammatory responses are too weak, the immune response can become dysregulated and excessively strong, and lead to host (patient) damage. IRS seems to reflect this latter scenario, where the balance between T_H1/T_H17 and T_H2/T_{reg} cells is disrupted in favor of proinflammatory responses. This may come about, for example, when receipt of antimicrobial therapy or withdrawal or reduction of immunosuppressive drugs causes a rapid reversal of pathogen- or drug-related immunosuppression, thereby promoting an abrupt and exaggerated shift in favor of proinflammatory responses. Other conditions may also be associated with a shift from T_H2/T_{reg} responses that restrain inflammation towards T_H1/T_H17 cells and generation of proinflammatory responses, including improvement in neutropenia status during the course of IFI and its treatment, reception of a bone marrow transplant (BMT), and the more subtle shifts in immunosuppression that occur during pregnancy and into the postpartum period.

In addition, certain fungal pathogens may have immunomodulatory effects that affect host response and that may contribute to development of IRS following initiation of antifungal therapy. In particular, a number of studies suggest *C neoformans* promotes an imbalance in favor of T_H2 cells over T_H1 cells, i.e., resulting in an anti-inflammatory environment and protection of the pathogen from the host immune response.^{7,13-17} When subsequent antifungal therapy eradicates *C neoformans*, the host may exhibit an exaggerated recovery of proinflammatory responses that leads to IRS.

Irrespective of fungal removal, immunomodulatory effects have been suggested for each of the major classes of antifungal drugs. As reviewed by Ben-Ami et al.,¹⁸ amphotericin deoxycholate itself has proinflammatory properties, and the various lipid formulations of amphotericin B each impact host immune cells to modulate immune response, generally in the direction of enhanced or proinflammatory responses. Triazole antifungals, and particularly fluconazole

and the structurally similar voriconazole, have been linked with enhanced activity of phagocytes, monocytes, macrophages, and neutrophils against *Candida albicans*.¹⁸ More recently, echinocandins have also been reported to exhibit immunomodulatory effects, including a possible disruption of the genetic network that protects β -glucan and other fungal cell components from attack by host immune cells under normal circumstances.¹⁸ It now appears that echinocandins may “unmask” β -glucan, allowing it to be detected and subsequently attacked by host immune cells. While generally beneficial, this echinocandin effect may also contribute to an exaggerated proinflammatory response and IRS under certain circumstances. However, from another perspective, echinocandins primarily work by inhibiting β -glucan synthesis, meaning they may ultimately remove β -glucan as an immunomodulator or source for immunologic reaction—thereby lowering risk of IRS.

At-Risk Populations and Risk Factors

IRS has now been described in several patient populations who appear to be at-risk for the condition. These include individuals infected with HIV who receive ART, solid-organ transplant recipients, neutropenic hosts, pregnant/postpartum women, and patients with chronic inflammatory diseases treated with tumor necrosis factor- α (TNF- α) inhibitors.^{2,3,7,8,19} Whatever the differences, in each of these groups, IRS appears to be the result of an exaggerated and apparently dysregulated proinflammatory response following restoration of immunity in a previously immunocompromised patient.⁷

ART Treatment in Patients with HIV. IRS has been described in as many as 30% to 32% of patients with HIV who are coinfecting with *C neoformans*, *Mycobacterium tuberculosis*, or *Mycobacterium avium* complex following treatment with highly active ART (HAART).^{20,21} In a recent systematic review and meta-analysis by Müller et al.²² of the development of IRS in patients with AIDS-defining illnesses starting ART, IRS developed in 37.7% of patients with cytomegalovirus (CMV) retinitis, 19.5% with cryptococcal meningitis, 15.7% with tuberculosis, 16.7% with multifocal leukoencephalopathy, and 6.4% with Kaposi sarcoma. The mortality rate for patients with any type of IRS was 4.5%, with the highest rate in patients with cryptococcal meningitis (20.8%).

The overriding risk factors for IRS in individuals infected with HIV are opportunistic infection (active or subclinical) or the presence of nonviable pathogens and/or their antigens, and initiation of ART or HAART, particularly in antiretroviral drug-naïve patients.^{2,20} Other risk factors include a low baseline CD4 T-cell count ($<50 \times 10^6$ cells/L)/advanced immunodeficiency at start of HAART, HAART initiated in close proximity to opportunistic infection diagnosis, and a more rapid fall in HIV-1 RNA level in response to HAART.^{20,22-26} In addition, patients coinfecting with HIV and *C neoformans* who develop *C neoformans*-related IRS have a higher CSF opening pressure, glucose levels, and WBC counts

compared with patients with typical HIV-associated *C neoformans* meningitis.²¹ It has been suggested that the patients with HIV at highest risk for IRS are those with the combination of disseminated infection, low baseline CD4 T-cell count, and early initiation of ART.²

Solid-Organ Transplant Recipients. IRS in recipients of solid-organ transplants is most commonly associated with *C neoformans* infection of the central nervous system (CNS), and more rarely with *M tuberculosis* or CMV infection (reviewed by Sun and Singh⁸). IRS has also been reported in a renal transplant recipient with polyoma BK virus infection.²⁷ An estimated 5-11% of solid-organ transplant recipients with cryptococcosis develop IRS,^{8,28-30} which is typically associated with initiation of effective antifungal therapy and/or cessation or significant reduction in immunosuppressive therapy.^{8,28} In a study by Singh et al.,²⁸ IRS-like illness developed in 4.8% of solid-organ transplant recipients with *C neoformans* infection a median of 5.5 weeks after starting appropriate antifungal therapy. Patients who developed an IRS-like entity were also more likely to have received more-potent immunosuppression (tacrolimus, MMF, and prednisone) ($P = 0.007$). The authors proposed that withdrawal or reduction of potent iatrogenic immunosuppression, together with initiation of antifungal therapy and removal of pathogen-related immunosuppression, probably causes a shift from T_H2 - to T_H1 -dominated immune status and a proinflammatory environment that increases risk of IRS in this patient population.

In addition to other inflammatory consequences, development of IRS in solid-organ transplant recipients also heightens the risk of allograft rejection and loss. For example, Singh et al.²⁹ reported that allograft loss was significantly greater in kidney transplant recipients with *C neoformans*-associated IRS than in kidney transplant recipients with *C neoformans* who did not develop IRS (66% vs. 6%, $P = 0.012$). A Kaplan-Meier analysis also showed the probability of allograft survival was lower for those patients who developed IRS versus those who did not ($P = 0.0004$). Interestingly, T_H1 and T_H17 cells are the primary mediators of allograft rejection, whereas T_H2 and T_{reg} protect against rejection (reviewed by Sun and Singh⁸). Similarly, immunosuppressive agents used to prevent allograft rejection shift the balance in favor of T_H2/T_{reg} cells versus T_H1/T_H17 cells.⁸ Hence, immune factors associated with IRS are the same as those associated with allograft rejection, suggesting a similar pathophysiologic basis for IRS and allograft rejection in solid-organ transplant recipients.

Returning to case 1, a number of additional observations can now be made. The patient presented with *C neoformans* infection of the CNS while on a potent immunosuppressive maintenance regimen and after prior treatment of an acute rejection episode with high-dose corticosteroids and muromonab-CD3. Presumably these treatments lowered the patient's immune status (created an imbalance in favor of T_H2/T_{reg} over T_H1/T_H17), thereby enabling the *C neoformans* infection to establish. The infection and immuno-

modulatory effects of *C neoformans* presumably caused further immunosuppression. The patient was treated with antifungal therapy and a reduction in immunosuppressive therapy (MMF discontinuation and lowering of tacrolimus and prednisone doses), which appeared to effectively eradicate *C neoformans*, based on the negative cultures and improvement in infection-related signs and symptoms. The successful removal of the pathogen and lowering of immunosuppressive therapy presumably enabled the patient's immune system to reconstitute, promoting a more proinflammatory versus anti-inflammatory environment. However, it appears the immune restoration became excessive, resulting in IRS. This would explain the subsequent onset of signs and symptoms consistent with an inflammatory process, together with negative cultures for *C neoformans* or other pathogens and failure to respond to subsequent antifungal therapy, together with allograft loss.

Patients with Neutropenia. A number of small studies or case reports have now described patients with neutropenia and invasive pulmonary aspergillosis who exhibited worsening of signs and symptoms of the disease during recovery of neutrophil count.³¹⁻³⁶ In some cases the patients died. All patients in these reports had neutropenia and a hematologic cancer that was treated with chemotherapy or a hematopoietic stem cell transplant (HSCT). A particularly instructive study was carried out by Miceli et al.,³⁴ in which they showed clinical and radiologic pulmonary deterioration in 19 neutropenic patients with pulmonary aspergillosis that coincided with neutrophil recovery and normalization of serum galactomannan levels. Serum galactomannan level is considered a marker for invasive aspergillosis, and generally correlates with outcome of invasive aspergillosis.^{37,38} Normalization of serum galactomannan is indicative of microbiologic response, i.e., eradication of *Aspergillus* species. Consistent with this conclusion, autopsies of three of the patients who died during the first months provided no evidence of aspergillosis.³⁴ The other 16 patients showed complete clinical response and survival at 3 months despite no change in antifungal therapy. The authors proposed that the clinical and radiographic deterioration in these patients that occurred during neutrophil recovery and serum galactomannan normalization was due to IRS, and proposed using serial galactomannan testing to distinguish between progressive aspergillosis and IRS, and guide management strategies.

Antinori et al.³² recently suggested that "clinical and radiological worsening of pulmonary invasive aspergillosis coincident with a robust decline of serum galactomannan values and rising neutrophil counts should be interpreted as (IRS) and should not require changes in antifungal therapy." Another study by Todeschini et al.³⁶ showed that rapid recovery (normalization of absolute neutrophil count within 5 days) may increase the risk of mortality in neutropenic patients with invasive pulmonary aspergillosis.

A limited number of reports have also described what appears to be IRS in adult and pediatric patients with neutropenia and hepatosplenic candidiasis.^{39,40} Consistent with

IRS, the patients in these reports exhibited signs and symptoms of inflammatory processes (or hepatosplenic candidiasis) coincident with rapid recovery of neutrophil count and that persisted despite antifungal therapy. Moreover, the conditions improved or eventually resolved after initiation of anti-inflammatory (corticosteroid) therapy. Taken together with the cases of IRS associated with pulmonary aspergillosis just described, the findings highlight the fact that clinicians need to be careful when using radiologic tests that detect signs consistent with either IRS or persistent infection. Other factors need to be considered when determining the most likely cause of the current patient presentation, which will affect treatment decisions. Echinocandins have demonstrated effectiveness in the treatment of less common forms of invasive candidiasis, such as hepatosplenic candidiasis.⁴¹ One might speculate that echinocandins might be optimal first-line therapy for hepatosplenic candidiasis, because disruption of β -glucan synthesis might remove this cell wall component as an immune modulator and potential source of IRS.

IRS after Pregnancy. Pregnancy is a state associated with relative immunosuppression, which enables the pregnancy to be carried to term without the mother's immune system attacking the developing embryo or fetus. T_H2 and T_H3 responses are enhanced and T_H1 responses suppressed during pregnancy (reviewed by Singh and Perfect¹⁹). During the postpartum period, T_H1 responses are restored, and this can trigger IRS in some women. Autoimmune disorders like rheumatoid arthritis and multiple sclerosis, among others, tend to improve during pregnancy before flaring up again during the postpartum period.¹⁹ Moreover, fungal (*C neoformans*), bacterial (*M tuberculosis* and *Mycobacterium leprae*), and viral (hepatitis B or C virus) infections have been reported to activate from a latent state and produce disease during the postpartum period of previously infected women.¹⁹ With respect to *C neoformans*, one early report described a young woman with chronic cryptococcal meningitis of approximately 16 years' duration who experienced a worsening after her second pregnancy and eventually died.⁴² Another report described the case of an aboriginal woman who developed *C gattii* meningitis with markedly elevated intracranial pressure during the postpartum period.⁴³ The patient had a poor response to antifungal therapy, and developed a paradoxical inflammatory reaction during treatment that was consistent with IRS.

TNF- α Inhibitor Recipients. TNF plays a key role in promoting inflammation and initiating other host responses to infection.⁴⁴ Conversely, TNF antagonists (adalimumab, etanercept, infliximab) are drugs that have been developed for the treatment of various chronic inflammatory diseases, and each carries a warning about being associated with increased risk of serious infections, including IFIs, tuberculosis, and bacterial sepsis, among others.⁴⁵⁻⁴⁷ IFIs associated with TNF antagonist therapy include histoplasmosis, invasive aspergillosis, cryptococcosis, candidiasis, and pneumocystosis.⁴⁸⁻⁵⁰

Since TNF blockade is stopped if an infection occurs, and antimicrobial treatment is initiated, IRS must be considered under conditions such as increasing clinical symptoms and radiograph abnormalities despite appropriate antimicrobial therapy. Infiltrates may actually get worse before they get better. TNF- α inhibitor discontinuation has recently been associated with IRS in a patient with cryptococcal pneumonia⁵¹ and another with tuberculosis.⁵²

Immune Reconstitution: the Good, the Bad, and the Ugly

Immune reconstitution can be good, bad, or simply ugly. It is good because a well-functioning host immune system is necessary for successful removal of fungal (and other microbial) infections over the long term. In fact, various immune-modulating strategies intended to "rev up" or boost the host immune system have been devised or are under investigation for the treatment of IFIs.⁵³⁻⁵⁵ One example is the 2004 study by Pappas et al.⁵⁶ that compared 2 different doses of interferon (IFN)- $\gamma1\beta$ (100 or 200 μ g, 3 times weekly for 10 weeks) or placebo as adjunctive therapy to standard antifungal treatment (amphotericin B \pm flucytosine, followed by fluconazole) in patients with AIDS-related acute cryptococcal meningitis. IFN- $\gamma1\beta$ is an endogenous cytokine that stimulates several host effector cells to boost T_H1 responsiveness. The results showed IFN- $\gamma1\beta$ was well tolerated and that there was a nonsignificant trend for improved combined mycologic and clinical success in IFN- $\gamma1\beta$ - versus placebo-treated patients (26% vs. 8%, $P = 0.078$). However, the key point here is the recognition that improved host responses can be a good thing that aids in fungal removal. On the other hand, as the discussions in this article have made clear, an overzealous and dysregulated host immune response can be destructive to the host, rather than protective. A relatively fine balance must be achieved and maintained. When immune reconstitution overshoots, the results are bad for the host. This is particularly the case when areas like the lung or brain are involved. The results may not only be bad, they can be deadly. For example, a study by Todeschini et al.³⁶ of patients with neutropenia with invasive aspergillosis and severe pulmonary complications coincident with rapid neutrophil recovery reported that 5 of 8 patients with pulmonary complications died. Therefore, whether immune reconstitution is good or bad depends on whether a healthy balance of proinflammatory and anti-inflammatory processes is achieved and the host is benefited, or whether the inflammatory response becomes excessive and destructive to the host.

Lastly, immune reconstitution can just be ugly. As just discussed, there are currently no useful biomarkers or tests for determining whether the clinical deterioration and inflammatory responses in a patient with an IFI refractory to current antifungal therapy reflects IRS or progressive infection. Moreover, even if one is fairly confident the patient is experiencing the ill effects of IRS, there are no real guidelines for how best to manage the condition, including when

to start therapy. Furthermore, when to start HAART when initiating the treatment of cryptococcal meningitis to reduce the chance of IRS is imprecise. For example, should it be started 2 weeks or 10 weeks after initiating antifungal therapy, and for how long? Studies are beginning to explore the optimal time for HAART initiation following treatment of another opportunistic infection, but there is no clear conclusion at this time.²

Additional Issues for Consideration: Biomarkers/Tests of IRS

It would be helpful to have biomarkers to either aid in IRS diagnosis or predict who is likely to develop IRS. However, there are currently no well established biomarkers or precise tests to predict or diagnose IRS associated with opportunistic mycoses or, for that matter, with HIV infection—although a number of studies have begun to explore potential biomarkers of HIV-associated IRS.⁵⁷⁻⁶⁰ With respect to mycoses-associated IRS, there are various types of skin tests and lymphoproliferative assays that have been investigated as potential biomarkers or tests for IRS, but there is nothing at this stage that can determine whether a given patient does or does not have IRS. Prospective studies with comprehensive immunological markers are needed. This is an important point. Diagnosing and managing patients with possible IRS is critical for providing optimal patient care, but it is an art, not a science. It requires a focus on trying to determine where the patient's immunity currently is, what is happening with the inflammatory reaction, and whether there is biologic control of the microorganism. No test currently exists to assist in indicating whether the inflammatory responses that are apparent are due to an excess response of the host or progressive infection. This fact is what makes diagnosis of the refractory fungal infections so difficult.

Management

The first decision after diagnosing a patient with IRS is whether to treat that patient. Not all cases of IRS need to be treated, and many cases will subside without treatment or consequences. However, CNS fungal infections and IRS can be problematic if not treated quickly and appropriately. Moreover, the combination of a prolonged diagnosis and IRS can make for a CNS disease that is difficult to manage. A delay in diagnosis may allow host immune responses to expand beyond what is helpful, and contribute to worse outcomes.⁶¹ For example, Nguyen et al.⁶² recently reported that outcomes of CNS cryptococcosis can be poorer in nonimmunosuppressed individuals than in severely immunosuppressed HIV patients, suggesting that host immune responses may contribute to pathogenesis, and a more robust or elaborated immune responses may portend worse outcomes.

Optimal management of IRS in patients with HIV or immunocompromised without HIV is unclear at present.^{2,3,7,8} Various anti-inflammatory therapies have been utilized with some success in these patients, including (most

commonly) corticosteroids as well as nonsteroidal anti-inflammatory drugs (e.g., naproxen) and thalidomide.^{3,25,63-66} The optimal length of treatment with any of these anti-inflammatory agents is also unclear at present. Based on my clinical experience for very tough cases and those involving the CNS, I would suggest using the guidelines for corticosteroid (dexamethasone) treatment of tuberculosis meningitis provided by Thwaites et al. in 2004.⁶⁷ However, as noted in case 1, a second course of corticosteroids was required because of IRS relapse. Other agents that have been proposed as potential future therapies for IRS include TNF- α inhibitors and statins.⁸

Case 2

A 47-year-old man in good health presented with cough, fever, and shortness of breath after cleaning out his attic with lots of bird droppings. A chest x-ray identified a pulmonary nodule in the lung, and fine-needle aspirate of the nodule grew *C neoformans* in culture. The serum cryptococcal antigen titer was 1:256, and cryptococcal antigen was not identified in CSF. The patient was initiated on a 1-day regimen of liposomal amphotericin B, followed by daily fluconazole therapy. Five months later, the patient reappeared with shortness of breath. A fine-needle biopsy identified *Cryptococcus*, but it did not grow in culture. The patient was restarted on intravenous liposomal amphotericin B for 6 weeks, but the treatment was terminated early owing to line sepsis. The patient's serum is now negative for cryptococcal antigen, but his lung biopsy shows necrotizing granulomas with numerous yeasts and interstitial chronic inflammation with reactive mesothelial hyperplasia. All cultures are negative. Pulmonary function tests indicate mild restrictive lung disease with oxygen desaturation.

In this case, it is likely that the patient inhaled a large burden of cryptococci, which was treated, but symptoms that were likely related to IRS reappeared. The patient was restarted on another antifungal regimen with new complications when, in fact, anti-inflammatory drugs might have been the best treatment choice with the new symptoms after ruling out the presence of viable yeasts.

SEVERELY IMMUNOCOMPROMISED WITHOUT EFFECTIVE DEFENSES: IMBALANCE FAVORING THE PATHOGEN

At the opposite extreme from IRS, there are some patients with such weakened immune systems that they become a growth medium for a wide number of opportunistic pathogens. Rather than having problems with immune restoration and tissue damage due to an overly robust immune/inflammatory response, the immune function in these patients is simply too weak to assist in pathogen removal. Obviously, the management strategy is quite different between patients with IRS and those with prolonged heightened susceptibility to multiple microbial infections. The clinician should be aware of the possibility of either extreme when trying to decide what is happening in a particular patient with con-

tinued problems despite apparently appropriate antifungal therapy. These points on the “human Petri dish” are illustrated by the following 3 case studies:

Case 3

A 60-year-old woman with advanced chronic lymphocytic leukemia (CLL) began thigh injections with alemtuzumab (Campath-1H), and her neutrophil count increased, but her CD4 count drastically decreased. She developed skin lesions that were painless, nodular, and slightly pruritic (see Figure 1). Biopsies of these lesions showed microsporidiosis. Because microsporidian are fungi,^{68,69} and microsporidiosis (which is largely observed in patients with AIDS or others with severe immunodeficiency^{68, 70}) has been successfully treated with albendazole, the patient was given albendazole for 4 weeks. Her microsporidiosis improved and was eventually cleared, but her depressed CD4 count persisted, and 3 months later, she was diagnosed with invasive sinusitis and osteomyelitis with *Fusarium* and *Aspergillus*. She then received treatment with voriconazole, with good response for her invasive sinusitis and osteomyelitis. However, 3 months later, the patient presented with chronic pneumonia. A lesion in her right lung was resected, and an attempt was made to clean out the pulmonary artery, which had become completely blocked with a *Rhizopus* embolus.

This is a classic case of a patient with a severe underlying disease who becomes severely immunocompromised after alemtuzumab therapy for it, and who essentially becomes a “human Petri dish” for growth of one pathogenic organism after another. As one treatment effectively eradicates the disease-causing pathogen it was designed to treat, another pathogen steps in to produce its own problems, and so on.



Figure 1 Multiple, slightly raised pruritic lesion on the thigh.

The immunocompromised condition of the patient—owing to both her weakened underlying disease state and the immunosuppressive drug therapy—has basically created very fertile conditions for one after another pathogenic microorganism to take hold and cause multiple fungal infections either together or in sequence.

Alemtuzumab is a fully humanized monoclonal antibody directed against CD52 antigen, which is highly expressed on both normal and malignant lymphocytes (B and T), as well as being located on various other immune cells (monocytes, macrophages, eosinophils, natural killer cells, and dendritic cells).^{71,72} Alemtuzumab has been approved for single-agent treatment of B-cell CLL⁷³ and has demonstrated efficacy in both patients with untreated or refractory/relapsed CLL,^{71,72,74} hence its use in this patient. However, alemtuzumab also produces profound and sometimes prolonged decreases in lymphocytes and other immune cells,^{74,75} which increases risk of invasive fungal and other infections.⁷⁴⁻⁷⁸ It is likely that alemtuzumab therapy contributed to the microsporidiosis initially observed in this patient, as well as provided the immunological groundwork or general conditions for subsequent infections.

It is also critical the clinician understands that the risk of dual infections is increased when dealing with a profoundly immunocompromised patient. This can involve either dual fungal infections,⁷⁹ or a combination of fungal and bacterial,⁸⁰ fungal and viral,^{81,82} or bacterial and viral infection. For instance, a retrospective review of patients with candidemia treated at Wayne State University from 1992 to 2000 identified polymicrobial candidemia (bloodstream infection with multiple *Candida* species) in 5.2% (16 of 303) of the patients.⁸³ Patients with polymicrobial candidemia tended to be sicker nononcologic patients, with serious underlying comorbidities and prolonged hospitalization. All of them had intravenous catheters, had been treated with multiple antibiotics, and were heavily colonized with *Candida*. The mortality rate for these patients was high (43%), but similar to that observed in patients with monomicrobial candidemia. Another study carried out at Duke University Medical Center identified polymicrobial candidemia in 2.8% (28 of 1,000) of patients with candidemia, which was associated with a 50% mortality rate.⁸⁴ Other studies have demonstrated dual mould infections (*Fonsecaea pedrosoi* in the lung and *Emericella nidulans* in the brain)⁸⁵ and even dual endemic mycoses (*Cryptococcus* and *Histoplasma*; personal observation) in patients with severe immunodeficiency.

Clinicians should be aware of the possibility of dual infection when dealing with a severely immunocompromised patient who does not seem to be responding to therapy as expected, and be very aggressive in seeking a diagnosis. Any and all tools available at the clinician’s disposal should be used, as necessary, with a focus on trying to understand where the patient is in the disease process and what his or her potential immunity is. As mentioned earlier, these patients are like “human Petri dishes,” and even the microorganism and the drugs themselves can impact the process and affect what fungus (or other pathogenic mi-

crobe) emerges as a problem. With respect to the impact of antifungal therapy on the type of IFI observed, a number of studies have reported that voriconazole, used as antifungal prophylaxis or primary treatment for invasive aspergillosis, is associated with breakthrough mucormycosis or mucormycosis superinfection.⁸⁶⁻⁹⁸ Furthermore, *in vitro* studies and animal models of mucormycosis suggest that exposure of “zygomycetes” to voriconazole can increase their virulence.^{99,100} Voriconazole is a broad-spectrum triazole with limited activity against fungi belonging to the order Mucorales (“zygomycetes”), and the development of pulmonary mucormycosis in the case patient may have been related to the treatment of *Aspergillus/Fusarium*-related invasive sinusitis and osteomyelitis with voriconazole.

The clinician needs to keep in mind the potential impact of voriconazole on mucormycosis development, but also be aware that not all broad-spectrum triazoles are the same. Although not considered the drug of choice for mucormycosis, posaconazole has demonstrated efficacy when used either as primary therapy for mucormycosis^{101,102} or as salvage therapy in patients with mucormycosis resistant to other antifungals.¹⁰³⁻¹¹⁰

Cases 4 and 5: Fusariosis

Case 4 was a 60-year-old allogeneic BMT recipient with profound and prolonged neutropenia (<100 cells/mL of blood) and erythema around a central line. Blood cultures were positive for *Fusarium solani* with the development of multiple skin lesions (Figure 2). The patient was treated with voriconazole and liposomal amphotericin B, without



Figure 2 Patient had severe neutropenia and positive blood cultures for *Fusarium solani* when these multiple lesions appeared over several days.



Figure 3 Surgical debridement of case patient's abdomen and chest.

therapeutic effect, and died 5 days later with concomitant pulmonary disease. At no point in the process did the patient show recovery of neutrophil count or any other signs of improved immune function.

Case 5 was a 2-year-old with a BMT for Hurler syndrome. He developed a subcutaneous infection at the catheter site that grew *Fusarium solani* in culture. His immune system exhibited signs of reconstitution, but the infection spread to the fingers, elbow, skull, and tibia. CT scans of the lungs, brain, liver, and spleen were all negative. Multimodal therapy was initiated, including antifungal therapy with liposomal amphotericin B (10 mg/kg/day) and voriconazole (12 mg/kg/day, titrated to a drug level of 1.6 µg/mL), surgical debridement, and silver impregnated packing and daily packing of the wound with gauze soaked in 0.02% polyhexamethylene-biguanide. Figure 3 illustrates the major surgical debridement of the patient's abdomen and chest. In addition, the patient received treatment with granulocyte/macrophage colony-stimulating factor (GM-CSF). Over a 7-month period with continuation of antifungal therapy, the lesions almost completely cleared and the wound closed. Liposomal amphotericin B was discontinued, but lesions reoccurred in the skin and tibia and elbow 3 to 4 weeks after discontinuation. Additional surgery was performed and treatment with liposomal amphotericin B was restarted and continued for another 2 months. The patient responded well, and at the time of this report was a healthy 6-year-old first grader cured of infection.

The first case of fusariosis occurs too frequently and can be very disheartening to the clinician in charge of the patient's care. It represents a high burden of organisms with delayed or inadequate immune reconstitution. This is a prescription for failure even with antifungal treatment. There is a tendency to think the situation is hopeless with these aggressive mould infections but as the second fusariosis case illustrates, successful outcomes can be achieved in severely immunocompromised patients even with inva-

sive fusariosis. The key is to never give up, to pay close attention to the patient, and to see if the patient's immunity can help. The clinician needs to remain aggressive, using every available tool at his or her disposal. High-dose liposomal amphotericin B, voriconazole, and adjunctive therapies, such as surgical debridement of the infected tissue and GM-CSF, are well recognized clinical tools for the management of fusariosis.¹¹¹ Importantly, unlike the immune system of the patient in case 4, which never reconstituted, the bone marrow transplant was successfully reconstituted in the patient in case 5, and the patient's immune function improved. The improved immune function, together with appropriate multimodal therapy, contributed to a successful outcome.

Moving beyond fusariosis to speak more generally about seriously ill patients with impaired immune systems and IFIs, clinicians may be able to generally predict outcomes based on the underlying disease of the patient. For example, a 2001 literature review of aspergillosis case-fatality rate (CFR) by Lin et al.¹¹² demonstrated substantial differences in CFR based on the underlying disease or condition of the patient population, with the lowest rates for lung or heart and lung transplant recipients (45%) or those with leukemia or lymphoma (49%), and highest rates for HIV/AIDS patients (86%) and BMT recipients (87%). The infection site was shown to be another important predictor of fatality due to aspergillosis, with lowest rates for sinusitis (25%) or localized disease (30%) and highest for those with CNS or disseminated aspergillosis (88%).¹¹² Disseminated cryptococcosis in cancer patients seems to be associated with particularly poor prognosis. A 1977 and 1992 study at Sloan-Kettering Cancer Center by Kaplan et al.¹¹³ and White et al.¹¹⁴ showed a particularly poor outcome with cryptococcosis and neoplasm. Investigators at MD Anderson Cancer Center more recently reported a better outcome in patients with cryptococcosis and an underlying neoplasm, but still reported a substantial mortality rate.¹¹⁵ The dire outlook for this patient population probably reflects the fact that the patients are at the end-stage of their underlying disease when cryptococcosis appears, i.e., progression of the cancer to a very late stage is probably what allows the disseminated cryptococcosis to occur in the first place. These examples clearly illustrate that with IFIs a major determining factor to outcome is the control of the underlying disease and immune state. It must be calculated into any treatment strategy.

SUMMARY

Managing immunocompromised patients who appear refractory to appropriate antifungal therapy can be a challenging process. The development of new or worsening clinical symptoms and signs consistent with an inflammatory process—after an apparent good response to therapy—may represent IRS, or it may represent treatment failure and progression of the initial infection, or a new infection. Given that there are limited tools for diagnosing IRS, the

clinician must use all his or her skills to practice the art of medicine. Using all available tools and information, the clinician has to try to discern the current state of the patient's immunity, and whether the problem at hand represents an overexuberant host immune response or something else. In real-world clinical practice, a patient's immunity may move back and forth during the course of treatment, from an initial immunosuppressed state to a restored and sometimes excessively strong immune response. At first the focus is on eradicating the offending pathogen, but once that is accomplished, the patient's immune system may recover in ways that surprise us. But it shouldn't, because we should be on guard for potential IRS. Similarly, we must be prepared to manage those patients who are so immunocompromised that they are subject to one opportunistic infection after another, and sometimes simultaneously. Early diagnosis is key to handling both of these situations, and when appropriate, prophylaxis may be the best approach in high-risk patients.

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